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LabLink

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Bureau of Laboratories

"Quality Laboratory Science for Healthier People and Communities"

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Fluoroquinolones No Longer Recommended for Treatment of Gonococcal Infections

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On April 13, the Centers for Disease Control and Prevention announced that the resistance of *Neisseria gonorrhoeae* (GC) to fluoroquinolones has reached sufficient levels across the United States that this class of antibiotics should no longer be used to treat infection with this agent. (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5614a3.htm) This development leaves the clinicians with limited options for treatment of GC and concern among public health officials that the increased cost of reliably effective treatments could seriously consume already limited sexually transmitted disease program budgets.

Quinolone-resistant *Neisseria gonorrhoeae* (QRNG) appears to have emerged in Michigan in 2003, when isolates were recovered in culture from 11 patients with no history of out-of state travel. A surveillance network was established including six hospital laboratories and five local health departments, which routinely performed genital culture that yielded an isolate for susceptibility testing. This sampling of isolates in Michigan has shown a continual increase in resistance, from 0.5% in 2002 to 4.8% in 2006.

However, monitoring for GC resistance to fluoroquinolone or other antimicrobial agents is challenging because most of the testing for GC is now routinely performed using nucleic acid amplification tests (NAATs) which does not yield a viable isolate. The MDCH Bureau of Laboratories (BOL) worked with the surveillance network and other partners to develop and evaluate an assay to detect resistant GC genotypes in residual NAAT specimens. Application of this assay to a random sample of GC-positive, NAAT specimens from public health labs across the state started in January 2007. Preliminary data suggests that resistance rates are between 4 and 5%. These findings indicate that resistance is clearly established in Michigan and support the CDC recommendation to use alternatives to the quinolones in treating *N. gonorrhoeae*.

The April announcement from CDC encourages susceptibility testing of isolates from patients who fail treatment with therapeutic alternatives to quinolones. MDCH is adapting its susceptibility testing protocol to reflect the CDC recommendations and will no longer report results for ciprofloxacin. While the minimum inhibitory concentration (MIC) values for *N. gonorrhoeae* against cephalosporins appear to be increasing, no resistant strains have yet been isolated. This limits the capability to develop a molecular assay to detect such resistance. The medical community will have to rely upon traditional susceptibility tests.

Michigan Department of Community Health (MDCH) Bureau of Laboratories (BOL) urges clinical colleagues to share the MMWR with the medical staff in their communities. Under the new recommendations, culture, rather than molecular-based testing, should be used when patients appear to have treatment failure. Please contact Dr. James Rudrik, Microbiology Section Manager at 517-335-9641 or rudrikj@michigan.gov with any questions or concerns.

FUN FUNGI.....

Microsporum nanum vs. *Trichothecium* spp.

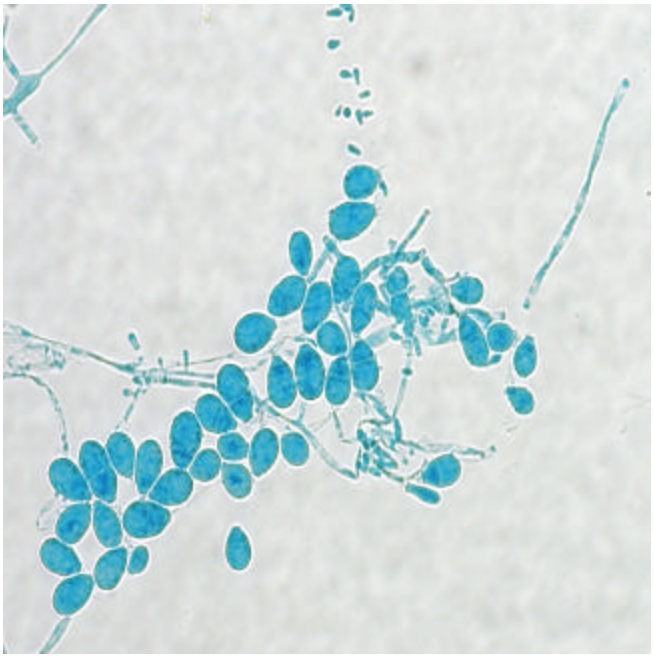
Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/Mycology Unit

Microsporum nanum is a geophilic and zoophilic fungus that is the principle cause of tinea in pigs. It is also a rare cause of tinea corporis and tinea capitis in humans. Human infection typically occurs from direct contact with infected pigs or fomites.

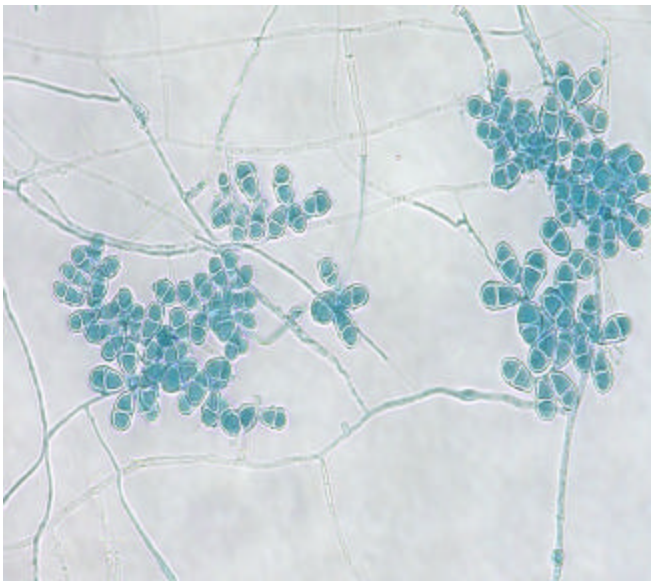
Colony growth is moderately rapid, cottony to powdery or suede-like and may have shallow furrows. The surface color is white to buff with a red-brown reverse. Microscopically, *M. nanum* produces numerous macroconidia that are characteristically pear or egg shaped and one to three celled (typically two celled). The macroconidia are thin walled with flat basal scars and may be smooth walled or finely roughened. Microconidia, if present, occur in small numbers and are clavate in shape. Hair perforation and urease tests are both positive. *Microsporum nanum* differs from *Trichothecium* spp. in that it produces solitary macroconidia on the ends of short, indistinct conidiophores that do not form in clusters.

***Trichothecium* spp.** are geophilic fungi with a worldwide distribution, most commonly found on decaying plant material. The only species reported with any frequency is *Trichothecium roseum*.

Trichothecium spp. form fast-growing colonies that are dusty pink or pale rose in color. Microscopically, the conidia are thick-walled, two-celled and have truncate bases. The upper cell is often slightly larger than the bottom cell. Un-branched conidiophores form imbricate (overlapping), zigzag chains of ellipsoidal conidia, giving the appearance of conidia in clusters.



Microsporum nanum



***Trichothecium* spp.**

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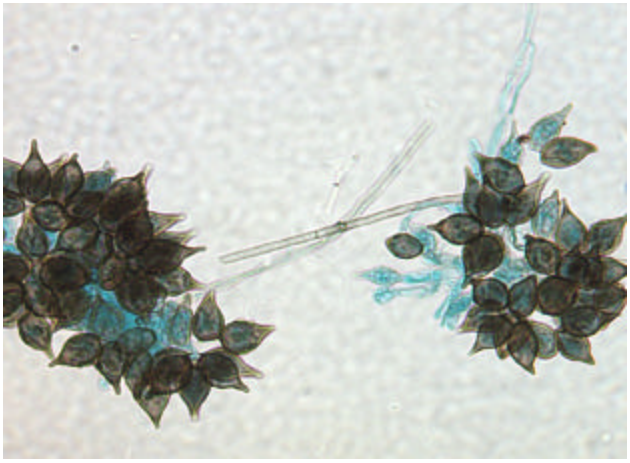
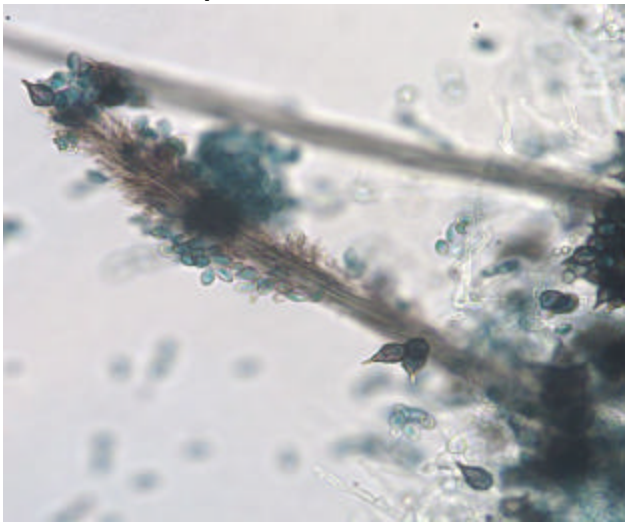
Last Issues Picture Quiz Answer:



Echinobotryum* Synanamorph of *Cephalotrichum stemonitis

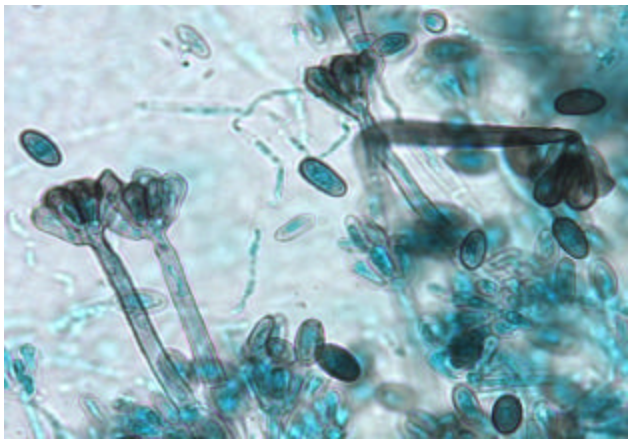


***Cephalotrichum stemonitis* with *Echinobotryum* Synanamorph Present**

Theses are pictures of *Cephalotrichum stemonitis* with an *Echinobotryum* synanamorph present. *C. stemonitis* is also known by the name *Doratomyces stemonitis*. This mould is called *Cephalotrichum* or *Doratomyces* depending on which reference is used. The genus name is undergoing a taxonomic debate and the matter is not yet resolved.

Cephalotrichum stemonitis is found worldwide in wood, dung and soil. Colonies are at first grey but become black with age. Microscopically, dematiaceous conidiophores form a synnemata with a fertile spore bearing head. The head is elongate and feathery, comprised of a central axis of hyphae, which bears numerous annellophores. The annellophores are short, inflated, and sometimes produced long chains of spores. The *Echinobotryum* synanamorph is also present. This is represented by flame shaped, dematiaceous aleuriospores formed directly on the sides of the hyphae or on short pedicels. These aleuriospores have a truncate base and are born singly or in groups. These roughened spores with an apical beak are characteristic of this synanamorph.

This Issues Picture Quiz: What Mould is this?



Hint: It causes hysteria due to media reports.

2007 Survey Results from Michigan Clinical Laboratories On Culture Protocols for *E. coli* O157

John Dyke, Ph.D.
Bureau of Laboratories

The national recall of *E. coli* O157 contaminated spinach and the health impact of this contamination, brings to light the important role laboratories play in identifying public health issues. The involvement of this agent in food-associated outbreaks in the last several years has been significant. As a result of the awareness of the disease severity and potential widespread distribution of *E. coli* O157:H7, clinical laboratory submitters are requesting rapid identification of this agent.

In 2001, the MDCH BOL conducted a survey of Michigan clinical laboratories to determine the practices for the recovery *E. coli* O157 from clinical specimens. Since that time, new diagnostic products have been developed and testing guidelines published (MMWR 29, 2006/55 (38); 1042). In addition, surveillance data over the last year has shown the emergence of non-O157 strains associated with food-associated outbreaks. Impact of these developments on clinical laboratories practices was again important to determine.

A questionnaire similar to that used in 2001 was administered to allow for comparison of results with the previous survey. The survey was sent to 123 clinical laboratories. Of the surveys sent, 69 (56%) were returned. In 2001, 102 (83%) laboratories responded to the survey. Despite the drop in respondents as a possible source of bias, important conclusions can be drawn by comparing results of the two surveys.

In the 2001 survey, 24 laboratories (23%) responded that they did not provide on-site testing services compared to 18 (26%) in 2007. However, the respondents not providing on-site testing from both surveys indicated that specimens for *E. coli* O157 culture would be sent to a reference laboratory.

Laboratories that had on-site services varied in criteria used to determine when specimens would be examined for *E. coli* O157. However, these selection criteria were similar in both surveys. Just over 50% of the laboratories examine for *E. coli* O157 on all stools submitted for culture. About 20% used bloody stools as examination criteria and 20% tested stools for *E. coli* O157 only when it was specifically ordered by the physician. A seasonal criterion was used by a limited number of laboratories.

All of the laboratories that cultured for *E. coli* O157 employed some type of selective/differential media. As in 2001, most laboratories continue to use MacConkey sorbitol agar as the screening media. About a third of the laboratories perform on-site serotyping of suspect colonies while the reminder sends isolates to a reference laboratory for confirmation and serotyping.

The availability of rapid, non-culture assays that detect shiga toxins continues to be available in a limited number of non-reference laboratories. The lack of commercial assay reagents has been a limiting factor; however, reagents may soon be available pending FDA approval.

All but one laboratory responding to the 2007 survey is sending suspected isolates to the MDCH. These isolates are essential in the effort to conduct disease surveillance and to protect the health of Michigan's citizens. MDCH would like to thank the clinical laboratories for continued support and participation in this process.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

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Bureau of Laboratories
100 Year Anniversary Update

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In celebration of the Michigan Department of Community Health, Bureau of Laboratories (BOL) Anniversary, we are proud to announce that the 100 Year Anniversary Exhibit is currently being displayed in the Michigan Historical Museum Atrium. The exhibit displays an overview of 100 years of public health achievements in Michigan using a timeline, photos and various pieces of laboratory equipment. The exhibit will be hosted at the Museum until June 30, 2007. Please don't miss out! For more information regarding hours of operation or Museum location, call (517)-373-3559 or visit www.michigan.gov/museum.

To commemorate the anniversary, the BOL also participated in the Michigan Public Health Week Kick-Off Event at the State Capitol on April 17th. The 100-year anniversary display board illustrating accomplishments, as well as pictures from the past and present was featured.

For more information about upcoming centennial events, please do not forget to visit our web page. It contains a picture gallery from the above mentioned events and a Blog where former employees and citizens who have been touched by the work of the laboratory can chronicle and share memories and photos.

The Bureau of Laboratories - 100 Year Anniversary Webpage can be accessed by visiting www.michigan.gov/mdchlab and then selecting the *Bureau of Laboratories- 100-Year Anniversary* link.

Bureau of Laboratories Vision

The Bureau of Laboratories is a stronger, more diverse team within an integrated public health system. We utilize advanced technology and innovative leadership to provide comprehensive public health services in our dynamic global community.

Bureau of Laboratories Mission

We are dedicated to continuing leadership in providing quality laboratory science for healthier people and communities through partnerships, communication and technical innovation.

QUIRKY BUGS…
Serratia ficaria

Glenn Fink, MT (ASCP)
Reference Bacteriology Unit

Among the wide variety of isolates submitted to the reference bacteriology unit at MDCH for identification, are members of the family *Enterobacteriaceae*. The nomenclature and classification of the genera of this family continues to undergo changes. Some rarely encountered species and low probability identifications for some isolates present a challenge to clinical microbiologists. While some of these organisms may be unfamiliar, laboratorians must be aware that these species do occur in clinical specimens as potential transients or commensals. A recent submission for identification illustrates this fact.

The submitted isolate was an oxidase negative gram-negative rod from a blood culture on a 16 year-old male. No further history was available. Growth on MacConkey agar revealed colonies with a clear, wet periphery and a pinkish center. On blood agar, the colonies were shiny, wet, raised, circular, and entire with a transparent periphery and opaque whitish center. They were non-hemolytic and somewhat adherent. The organism was catalase positive and motile. A comprehensive enteric fermenter tube biochemical profile was performed and the isolate was identified with a 97% confidence level as *Serratia ficaria.*

Serratia ficaria was first recognized and identified by Grimont et. al. in 1979. Its name was derived from the fig tree species *Ficus carica*, as it plays an important role in the Smyrna-type figs-fig wasp ecosystem. In addition to figs and fig wasps, other natural reservoirs of this organism include common grass, market mushrooms, ants, and other insects. While the frequency in human clinical specimens is rare, it has been isolated from respiratory tract specimens, wounds, blood, bile and stool. It appears to be recovered from (at least transiently) the mouth, upper respiratory system and human intestinal flora, and its presence seems dependent on both environmental and climatic factors. Its role as the causative agent of opportunistic infections has become clearer in recent years as more reports surface. Its pathogenicity is probably low, as the course to recovery seems to be uncomplicated and speedy.

Serratia ficaria grows on MacConkey agar (see Figure 1), and is an oxidase negative, non-pigmented glucose fermenting gram-negative bacillus. It is catalase positive, nitrate reductase positive, esculin hydrolysis positive, and citrate positive, as well as positive for DNase and gelatinase at 25°C. The organism is negative for H_2S, urease, indole, tryptophan deaminase, and the decarboxylases. In addition to fermenting glucose, it also ferments xylose, mannitol, sucrose, maltose, arabinose, cellobiose, sorbitol, and trehalose. This organism is negative for dulcitol and malonate, and variable for adonitol, lactose, rhamnose, arabitol, inositol, raffinose, and melibiose. It is also variable for methyl red, Voges-Proskauer, and acetate. *Serratia ficaria* usually, but not always, has a potato-like or other vegetable matter odor, similar to *Serratia odorifera*.

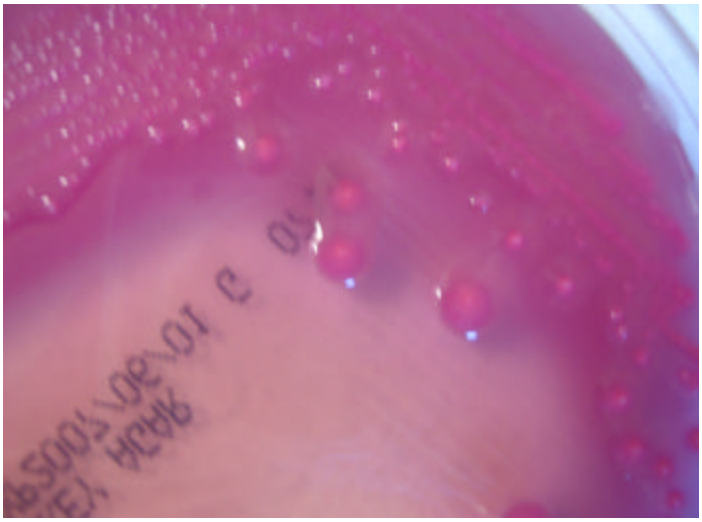
A variety of API 20E numerical profiles have been seen with this organism, including 1206773, 1206763, and 1204773. As with many of the unusual strains of *Enterobacteriaceae*, conventional tests are often required for a correct identification. The databases of various commercial systems were built on a relatively small number of available strains, and thus the ability of these systems to identify these organisms is tentative

at best. Some studies suggest the percent positivity is lower than previously published for these systems and misidentification could occur. A brief conventional biochemical profile for separation of *Serratia* species may be found in Table 1.

Because of the infrequent identification of this organism, susceptibility patterns are difficult to generate. In limited reports, *Serratia ficaria* appears to be sensitive to amikacin, gentamicin, tobramycin, piperacillin, piperacillin/tazobactam, and some cephalosporins (cefotaxime, ceftriaxone, ceftazidime). It appears intermediate or resistant to tetracycline, amoxicillin, ampicillin, loracarbef, chloramphenicol, rifampin, cefaclor, streptomycin, colistin, cefoxitin, and cephalothin.

The correct identification to the species level is becoming increasingly appreciated as susceptibility patterns suggest species-specific mechanisms for antibiotic resistance. Rarely encountered species and low probability identifications should always be sent to a reference laboratory for identification or confirmation. A brief history and the origin of the specimen are important for efficient and accurate identification.

Figure 1



***Serratia ficaria* – typical colonies on MacConkey agar**

Table 1. Biochemical Differentiation of Members of the Genus *Serratia*

Species	LDC	ODC	Mal	Arabinose	Rhamnose	Xylose	Sucrose	Adonitol	Sorbitol	Cellobiose
S. entomophila	-	-	-	-	-	V	+	-	-	-
S .ficaria	-	-	-	+	V	+	+	V	+	+
S. fonticola	+	+	V	+	V	V	V	+	+	-
S. liquefaciens group	+	+	-	+	V	+	+	-	+	-
S. marcescens	+	+	-	-	-	-	+	V	+	-
S. marcescens biogroup 1	V	V	-	-	-	-	+	V	+	-
S. odorifera biogroup 1	+	+	-	+	+	+	+	V	+	+
S. odorifera biogroup 2	+	-	-	+	+	+	-	V	+	+
S. plymuthica	-	-	-	+	-	+	+	-	V	V
S. rubidaea	V	-	+	+	-	+	+	+	-	+

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What's in Your Peanut Butter?

Patricia Somsel, Dr.P.H.
Division of Infectious Diseases

The recent outbreak of infection with *Salmonella* Tennessee, associated with peanut butter, illustrates the vast reach of the food processors that provide service for the food retailers in our nation. According to a Washington Post report (April 23, 2007, *FDA Aware of Dangers to Food*) a ConAgra plant, located in Augusta, GA, produces product under the name of Peter Pan and Great Value brands, the latter distributed to at least 70 foreign countries in addition to national distribution. The likely cause, ConAgra reported, was a roof leak and a malfunctioning sprinkler system that "activated dormant salmonella" in August of 2006, although a 2005 FDA reportedly indicates an "alleged episode of positive findings of salmonella in peanut butter in October 2004 at the firm."

The first isolates were submitted in August of 2006. Over 300 cases had been recognized in the United States by the time the source was identified as peanut butter and the products withdrawn from the market in February of 2007. The current case count (as of 4/25/07) is 563 cases from 47 states, with the last isolate submitted on 4/15/2007. The first Michigan case was submitted to the BOL in January 2007, followed by 13 more. This outbreak was unusual in a number of ways. The average age of 51 (range <1 – 95) was higher than normal, due to the evident preponderance of peanut butter in the diet of the elderly. Also of note, 35% of the isolates were recovered from urine cultures rather than from fecal cultures.

Recognition of this outbreak was greatly facilitated by the PulseNet System. Isolates submitted, primarily from clinical laboratories, are characterized by molecular means. The data submitted by each state is compiled in a central database, where states and CDC can match isolates. This database enhances epidemiologic investigations, allowing for early recognition of a common source outbreak and identification of the suspected source. The identification of the source of this outbreak was somewhat delayed because of the wide range of implicated products. Multiple sizes and forms of packaging, from two pound buckets to individual serving sizes, were contaminated.

The contribution of clinical laboratories to the recognition of this outbreak cannot be overstated. Public health officials have come to recognize that clinical microbiologists at benches across the nation and here in Michigan are the front line of detection of infectious diseases and essential players in the health of our communities.